Genetic Diversity Analysis of Cluster Bean [*Cyamopsis tetragonoloba* (L.) Taub] Genotypes Using RAPD and ISSR Markers

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ABSTRACT

In the present study, random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers were used to estimate genetic diversity and relationships among 35 cluster bean [*Cyamopsis tetragonoloba* (L.) Taub] genotypes. For RAPD analysis, 20 random primers were used which amplified 164 bands, 147 of which were polymorphic with an average polymorphism of 90.3%. The amplified products varied in size from 250 to 2,050 bp. For ISSR analysis, 10 primers were used which produced 105 bands, 102 of which were polymorphic (97%). The size of amplified bands ranged from 450 to 3,500 bp. The efficiency of primers in generating sufficient information for genetic diversity analysis was computed using discriminatory power (D_j), which ranged from 0.40 to 0.98 for RAPDs and 0.44 to 0.99 for ISSRs. Jaccard similarity coefficients were used to estimate the genotypic association with each other, which varied from 0.38 to 0.91 for RAPDs and from 0.20 to 0.88 for ISSRs. Cluster analysis indicated that all 35 genotypes could be distinguished by both RAPD and ISSR markers. Both of the methods (RAPD and ISSR) showed significant correlation (r= 0.69), implying their equal importance in cluster bean diversity analysis.

Keywords: Cluster analysis, Discrimination Power, Polymorphism, Similarity coefficient.

INTRODUCTION

Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub, family Leguminosae], commonly known as guar, has an important place in industry because of its seeds, which contain galactomannan rich endosperm. India and Pakistan export much of their guar crop to the United States and other countries in the form of partially processed endosperm material. World demand for guar has increased in recent years, leading to crop introductions in several countries (Undersander et al., 1991). Despite the great significance of this species, only few reports exist for the development of genomic resources in guar (Dhugga *et al*., 2004). Indian guar cultivars have been developed traditionally by selection, hybridization, and back crossing with locally adapted high-yielding lines. Therefore, attempts to analyze possible untapped genetic diversity become extremely essential for breeding and crop improvement.

Molecular markers have been applied widely in genetic analyses and breeding studies, as well as investigations of genetic diversity and relationships between cultivated species. These methods have several advantages over morphological markers, including high polymorphism rate and independent effects related to

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environmental conditions and the physiological stage of the plants. Several DNA marker systems are used in assessing genetic diversity of plants (Karp *et al*., 1997), the most commonly used marker systems are random amplified polymorphic DNA (RAPD) (Williams *et al*., 1990), restriction fragment length polymorphism (RFLP) (Soller and Beckmann, 1983), amplified fragment length polymorphism (AFLP) (Seehalak *et al*., 2006), inter simple sequence repeats (ISSRs) (Zietkiewicz *et al*., 1994) and microsatellites or simple sequence repeats (SSRs) (Becker and Heun, 1994).

Among all the molecular markers, random markers like RAPDs and ISSRs are most widely used because they are inexpensive, quick, simple and do not require sequence information (Williams *et al*., 1990; Zietkiewicz *et al*., 1994).

RAPD being a multi locus marker (Karp *et al*., 1997) with the simplest and fastest detection technology has been used for diversity analysis in several tree species (Vaishali *et al*., 2008; Khan *et al*., 2010) and many crop species (Demeke *et al*., 1992; Agarwal *et al*., 2008) including legumes (Kaga *et al*., 1996; Weder, 2002). However, RAPD poses some problems of reproducibility (Waugh and Powell, 1992). In contrast, ISSR markers are preferred over RAPD because of their long primer length, which perfectly bind to their target site and produce repeatable banding profiles. ISSRs have successfully been used in genetic diversity analysis of black gram and chick pea plants (Souframanien and Gopalakrishna, 2004; Rao *et al*., 2007) and both types of marker have been useful for identifying relationships at the cultivar and species level (Rao *et al*., 2007; Sharma *et al*., 2008).

Information regarding the extent and pattern of genetic variation in cluster bean is limited. Therefore, urgent efforts are required to improve yield and gum quality of cluster bean using conventional and biotechnological approaches. An assessment of the genetic diversity is an important first step to achieve this goal. Genetic diversity

analysis is crucial for breeders to better understand the evolutionary and genetic relationships among accessions, to select germplasm in a more systemic and effective fashion, and to develop strategies to incorporate useful diversity in their breeding programs (Paterson *et al*., 1991). Previous studies on characterization of cluster bean germplasm used phenotypic characters (Shabarish *et al*., 2012) or qualitative traits (Pathak *et al*., 2011). Biochemical marker studies using allozyme (Brahmi *et al*., 2004) have also been carried out. Among molecular markers, only RAPD has been used for genetic variability analysis (Punia *et al*., 2009; Pathak *et al*., 2010). Despite great industrial value, cluster bean has not been explored in detail which prompted us to undertake the present work with random molecular markers. This paper describes genetic diversity estimation among 35 elite genotypes of cluster bean, using RAPD and ISSR markers.

MATERIALS AND METHODS

Plant Material

For the present investigation, six major guar growing states of India i.e. Gujarat, Haryana, Madhya Pradesh, Punjab, Rajasthan, and Uttar Pradesh were selected for collection of seeds as the soil and climatic conditions of these states are favorable for production of cluster bean. (Table 1). Seeds of 35 genotypes mainly grown in these states and used for breeding purposes were collected. The seeds were grown in green house and healthy leaves from 10 random plants were collected and pooled for each genotype for the extraction of genomic DNA.

DNA Isolation

DNA was extracted from leaves of all the genotypes using the CTAB method of Doyle and Doyle (1990). The extracted DNA was

Table 1. Different genotypes of cluster bean collected from different states of India.

State	Genotypes
Rajasthan	RGC-197, RGC-936, RGC-986, RGC-1002, RGC-1003, RGC-1031, RGC-1017, RGC-1053, RGC-1066, RGC-1030, RGM-111, RGM-112, RGM-115, Bundel guar
Haryana	HG- 365, Priya- 151, HG- 353, HG- 563, HG-75, HG-890, FS-22
Gujarat	Swati-55, Jyoti- 555, Parasiya, Guara- 80
Uttar Pradesh	Pusa-Navbhar, Amul-51, Neelam-51, PNB, Krishna
Punjab	Jyoti-55, Local-selection, Ageta- 111
Madhya Pradesh	Pusa Selection I, Pusa Sadabhar

purified through RNAse treatment and tested on agarose gel for integrity. Purified DNA was quantified using Nano Drop (NanoDrop ND-1000 Version 3.1.1) spectrophotometer. After quantification, the DNA was diluted with TE buffer (Tris 10 mM and EDTA 1 mM, pH= 8.0) to a working concentration of 25 ng µl^{-1} for PCR analysis.

RAPD and ISSR analysis

For RAPD and ISSR analysis, only those primers were selected that showed reproducible and contrasting amplification patterns. Using this scale, finally, 20 RAPD and 10 ISSR primers were selected for the diversity analysis of the current set of cluster bean genotypes.

The RAPD and ISSR-PCR reactions were performed in a 25 µl reaction mixture containing 50 ng of template DNA, 0.2 mM dNTPs, 1X *Taq* buffer with 1.5 mM MgCl$_2$, 1U *Taq* DNA polymerase and 6 pmol of each primer. PCR amplification was performed in thermal cycler (PEQLAB, Germany) using the following amplification profile: initial denaturation of template DNA at 94°C for 4 minutes followed by 44 amplification cycles of denaturation at 94°C for 1 minute primer annealing at 37°C (RAPD) and 52–62°C (ISSR) for 1 minute and elongation at 72°C for 2 minutes followed by a final extension step at 72°C for 5 minutes.

The RAPD and ISSR-PCR products were resolved on 1.5% agarose gels with 1X TBE buffer containing ethidium bromide (0.5 µg ml^{-1}) and then photographed under UV with gel documentation system (Biorad).

Data Analysis

The RAPD and ISSR products were scored for the presence (1) and absence (0) of each primer-genotype combination. The pair wise genetic similarities among all pairs of samples were estimated with Jaccard coefficient (Jaccard, 1908) and similarity matrix was constructed. This matrix was subjected to un-weighted pair-group method for arithmetic average analysis (UPGMA) to generate dendrogram. All these computations were carried out using NTSYS-PC software version 2.02e (Rohlf, 1997).

The discriminatory power (D_j= 1-C_j) and confusion probability (C_j) of the jth assay (Tessier *et al*., 1999) were calculated according to the following equation:

$$C_j = \sum_{i=1}^{I} p_i (N_{pi} - 1)/N-1 \qquad (1)$$

Where, p_i represents the frequency of the ith pattern, N the sample size, and I is the total number of patterns generated by the jth assay. The confusion probability reflects the probability that two randomly chosen accessions share an identical pattern.

RESULTS

RAPD Analysis

In RAPD analysis, 20 RAPD primers generated a total of 164 amplicons across all the genotypes with an average of 8.2 bands/primer (Figure 1). The amplified
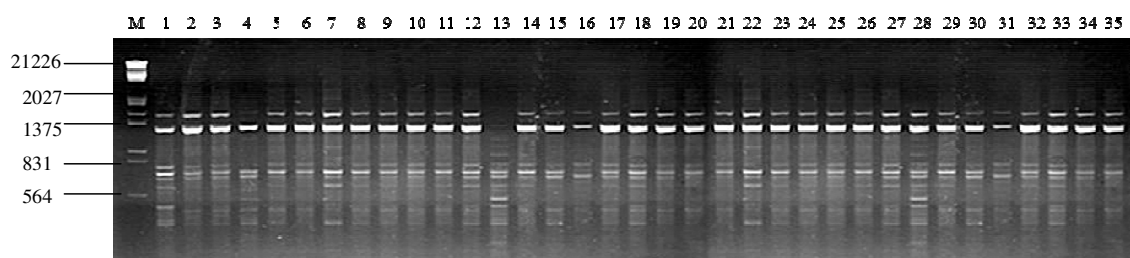
Figure 1. RAPD profiling of different genotypes of clusterbean (Lane M- Lambda DNA *EcoR1/HindIII* double digest, Lane 1-35- RAPD primer RP-7).

products ranged from 220 to 2,050 bp. Maximum number of 13 amplicons were produced by primer RP-44 and the lowest (3) by RP-15. Of the 164 amplified bands, 147 were found polymorphic with 89.63% polymorphism. Only 2 RAPD primers showed less than 75% polymorphism. The RAPD primer efficiency, which was calculated as discrimination power (D_j), ranged from 0.40 (RP-13 and RP-15) to 0.98 (RP-3) (Table 2).

RAPD Based Genetic Variability Analysis

Jaccard similarity coefficients among all the pair-wise combinations of accessions ranged from 0.38 to 0.91, with a mean genetic similarity of 0.69. Highest similarity (0.91) was observed between genotypes of Rajasthan (RGM-112 and RGM-115), while

Table 2. Total number of amplified fragments and number of polymorphic fragments generated by PCR using selected random decamers in 35 genotypes of cluster bean.

Primer	Sequence (5'-3')	Total bands	No. of polymorphic bands	% Polymorphism	Range of fragment size (bp)	Discrimination power (Dj)
RP-2	*TGCCGAGCTG*	9	9	100	450-1900	0.8
RP-3	*TGACCCCTCC*	9	7	77.7	250-2025	0.98
RP-5	*AGGGGTCTTG*	7	6	85.7	475-1350	0.65
RP-7	*GAAACGGGTG*	9	9	100	450-1400	0.94
RP-8	*GTGACGTAGG*	4	4	100	250-1200	0.71
RP-9	*CCGTCATTGG*	9	9	100	350-3000	0.95
RP-10	*GTGATCGCAG*	12	11	91.6	350-1580	0.92
RP-13	*CACCACCCAC*	10	6	60	250-1900	0.4
RP-15	*TTCCGAACCC*	3	3	100	850-1000	0.4
RP-19	*CAGGCGGCGT*	5	5	100	550-1000	0.87
RP-42	*AGTAGGGCAC*	7	4	57.1	250-930	0.11
RP-44	*GGACGCTTCA*	13	12	92.3	250-1900	0.95
RP-45	*GGGTAACGTG*	5	4	80	300-1700	0.41
RP-46	*AGTGCAGCCA*	12	10	83.3	450-1375	0.85
RP-48	*AGGGTGGGTG*	11	11	100	250-1350	0.91
RP-51	*GGGACGATGC*	10	8	80	350-1580	0.88
RP-54	*AGTGCGCTGA*	9	9	100	250-2000	0.88
RP-55	*CCGCGTCTTG*	6	6	100	250-1450	0.75
RP-57	*CCCCGAAGGT*	6	6	100	450-2025	0.49
RP-59	*GGCTAACCGA*	8	8	100	250-1580	0.68
	Total	164	147			
	Average	8.2	7.35	90.3		0.72

it was lowest (0.38) between RGC-1031 (Rajasthan) and Jyoti-555 (Gujarat). Maximum within- group similarity (84%) was observed for genotypes from Punjab, followed by Uttar Pradesh (77%) and Haryana (76%). The genotypes from Rajasthan and Madhya Pradesh showed 71 and 66% similarity, respectively, while genotypes of Gujarat showed minimum group similarity, i.e. 57%.

UPGMA-based dendrogram clustered all the genotypes into 9 main groups at average similarity value of 0.72 as cut-off mark. Cluster I consisted only of RGC-936 (Rajasthan). Cluster II sub clustered into two sub clusters, the first having genotypes of Rajasthan and Haryana, whereas the second sub cluster consisted only of one genotype of Haryana i.e. Priya-151. Cluster III included only one genotype of Haryana (HG-75). Cluster IV again divided into sub clusters, i.e. PNB (Uttar Pradesh). The other consisted of a mixture of 10 genotypes of different states from Uttar Pradesh, Punjab, and Gujarat. Cluster V, VI, VII, VIII, IX represented single genotypes from Gujarat (Parasiya and Jyoti-555), Madhya Pradesh, (Pusa-Sadabhar) and Rajasthan (RGC-1053 and RGC-1031), respectively.

ISSR Analysis

In case of ISSRs, only 10 primers that had high reproducibility were selected for diversity analysis (Figure 2). Based on all the primers and genotypes, a total of 105 band positions were obtained, which ranged

from 7 to 17 bands. The size of amplified products ranged from 450 bp to 3,500 bp. Out of 105 band positions, 102 were found to be polymorphic with 97% polymorphism (Table 3). The results showed that polymorphism index varied from 77.7 to 100% for different primers with a mean of 97% (Table 3). The highest polymorphism was observed with 8 primers IS-5, IS-7, IS-8, IS-14, IS-19, IS-21, IS-23, and IS-25, while it was at minimum for primer IS-6.

The number of bands produced with different repeat nucleotides were more with the GA-repeat based primers (IS-7 and IS-8) and AC-repeat primers (IS-19, IS-20 and IS-21), whereas primers having AG-repeats (IS-5 and IS-6) produced comparatively less numbers of bands. Similarly, the primers with GA-repeats, AC-repeats, and GT – repeats produced comparatively more polymorphism. No amplification was observed in primers having dinucleotide repeat (AT)n, (TC)n, (TA)n, (CT)n with T at the 5' end, tri-nucleotides repeats (ACC)n, (TGC)n and in primers with tetra-nucleotides repeats (CTAG)n and (TGCA)n. On the basis of these unique band profiles, the maximum discriminating power was obtained using primer IS-8 (0.99), while the minimum (0.44) was obtained with IS-19.

ISSR Based Genetic Variability Analysis

ISSR-based genetic similarity coefficients among all pair-wise combinations ranged between 0.20 and 0.88, with a mean value of
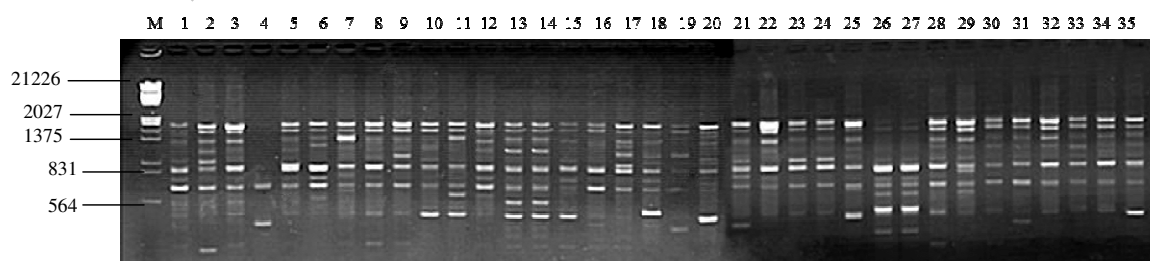

Figure 2. ISSR profiling of different genotypes of clusterbean (Lane M: Lambda DNA *EcoR1/HindIII* double digest, Lane 1-35: ISSR primer IS-7).

Table 3. Total number of amplified fragments and number of polymorphic fragments generated by PCR using ISSR primers in 35 genotypes of cluster bean.

Primer	Sequence	Tm (°C)	Total bands	No. of polymorphic bands	% Polymorphism	Range of fragment size (bp)	Discrimination power (Dj)
IS-5	$(AG)_8T$	50	8	8	100	560-3500	0.86
IS-6	$(AG)_8C$	52	9	7	77.7	600-1800	0.85
IS-7	$(GA)_8T$	50	17	17	100	560-3500	0.96
IS-8	$(GA)_8C$	52	13	13	100	650-3500	0.99
IS-14	$(GT)_8C$	54	5	5	100	1000-3000	0.84
IS-19	$(AC)_8T$	50	7	7	100	500-3000	0.44
IS-20	$(AC)_8C$	54	13	12	92.3	564-3000	0.94
IS-21	$(AC)_8G$	54	13	13	100	450-2800	0.98
IS-23	$(TG)_8C$	52	12	12	100	700-3000	0.97
IS-25	$(AGC)_6$	60	8	8	100	564-2500	0.78
	Total		105	102			
	Average		10.5	10.2	97		0.86

0.61. Maximum similarity coefficient (0.88) was observed between genotypes of Rajasthan (RGC-197 and RGM-112), while it was found minimum (0.20) between genotypes of Rajasthan (RGC-1031) and Haryana (HG-563). Maximum similarity of 85% was observed among genotypes from Punjab followed by 75% in Uttar Pradesh genotypes. Genotypes from Rajasthan showed 61% similarity while genotypes of Madhya Pradesh, Haryana and Gujarat showed 63, 58, and 42% similarity, respectively.

The ISSR-based dendrogram clustered all the genotypes into 7 groups at average similarity coefficient of 0.61 as cut-off value. The first group included genotypes of Rajasthan and Haryana while the second group included the remaining genotypes of Haryana, Gujarat, Uttar Pradesh and Punjab. Some genotypes like Pusa-Sadabhar (Madhya Pradesh), RGC-1053 and RGC-1031 (Rajasthan), HG-563 (Haryana), and Jyoti-555 (Gujarat) showed separate branches in dendrogram in cluster III, IV, V, VI and VII.

Comparative Analysis of RAPDs and ISSRs

ISSR markers were more efficient than the RAPD assay with regard to polymorphism

detection, as they detected 97% as compared to 90.3% for RAPD markers. Also, the average numbers of polymorphic bands per primer were more for ISSR (10.5) than for RAPD (8.2). The ISSR-based Jaccard similarity coefficients ranged from 0.44 to 0.99 while RAPD-based similarity coefficient ranged from 0.11 to 0.98. Similarity indices values based on both of the marker systems together ranged from 0.33 to 0.86. The product moment correlation (r) and the Mantel test statistic (Z) were calculated to measure the degree of relationship between the similarity matrices produced by RAPD and ISSR data, which were found significant (0.69).

Combined RAPD and ISSR Analysis

Due to high correlation among matrices derived from RAPDs and ISSRs, a combined dendrogram of 164 RAPD and 105 ISSR markers was constructed. The combined dendrogram represented virtual cluster to RAPD and ISSR. However, it was more inclined towards RAPD-based dendrogram. The Jaccard similarity coefficients in combined matrix ranged from 0.33 to 0.86. RGM-111 and RGM-112 showed the highest similarity index (0.86),

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while the genotypes RGC-1031 and Jyoti-555 showed the lowest similarity index (0.33). The mean similarity index was 0.66. In all the three dendrograms, Jyoti-555 was present in different cluster (Figure 3).

DISCUSSION

Owing to the unavailability of the next generation markers in cluster bean, the study was carried out with most commonly used
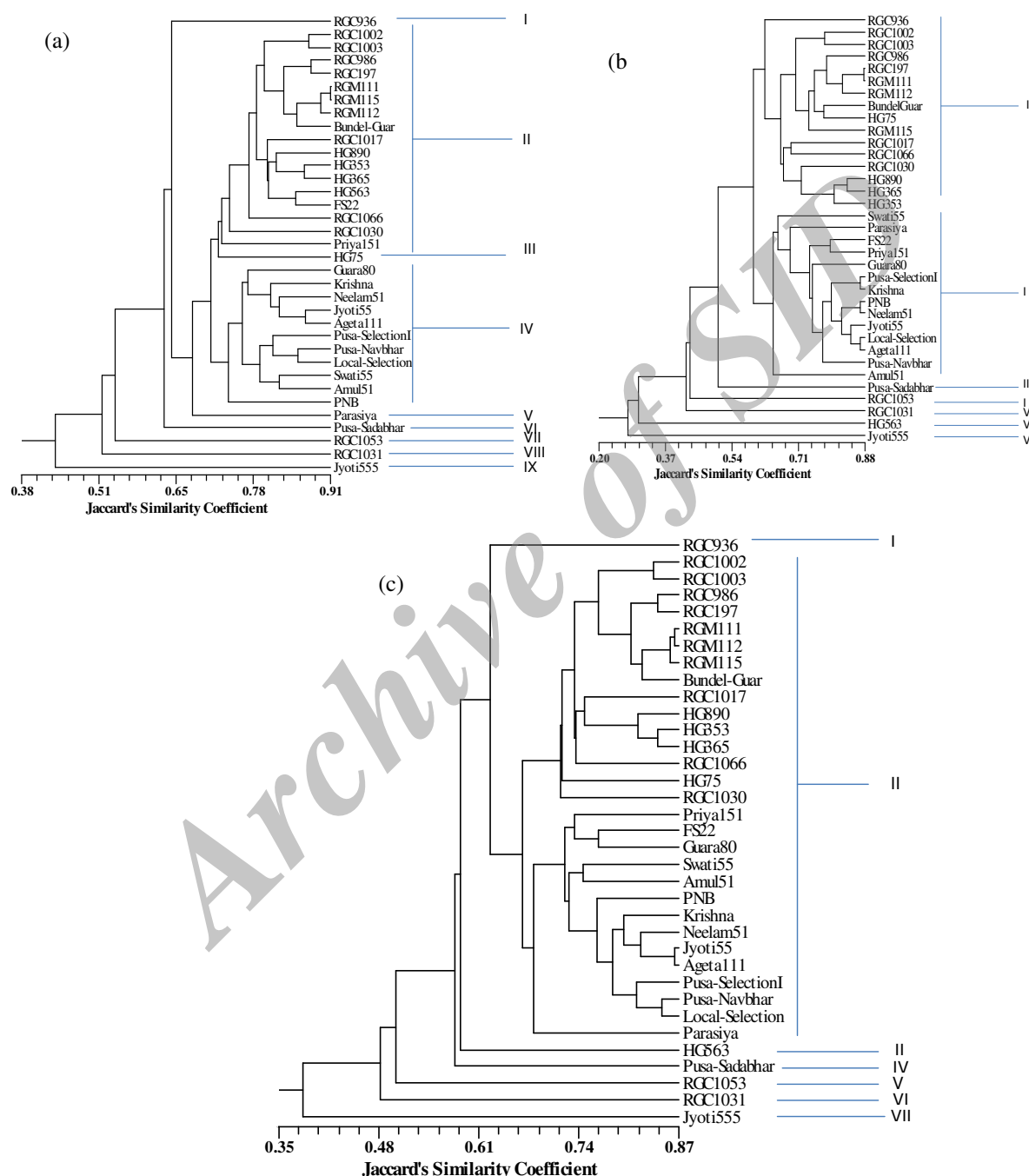

Figure 3. Dendrogram generated using UPGMA analysis, showing relationships between cluster bean genotypes using: (a) RAPD; (b) ISSR, and (c) Combined RAPD and ISSR data.

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random markers such as RAPD and ISSR. Both methods generated good diversity for the present set of cluster bean genotypes. Earlier studies also showed a large genetic variation among different genotypes of cluster bean using the RAPD (Punia *et al*., 2009; Pathak *et al*., 2010). In this study, ISSRs (97% polymorphism) were found to be more efficient over RAPDs (90.3% polymorphism) in estimating genetic diversity. Similar results have been obtained for several other crops, including wheat (Nagaoka and Ogihara, 1997) and groundnut (Raina *et al*., 2001). The greater ability of ISSRs over RAPD was due to semi-arbitrary nature and longer length, which increases annealing efficiency. The mean number of amplification fragments obtained with RAPD (8.2) was lower than that with ISSR (10.5). Therefore, the ISSR markers represent the more efficient marker system because of their capacity to generate several informative bands within a single amplification reaction. Similar results have been reported in cashew by Thimmappaiah *et al*. (2009). In fact, ISSR exhibits a higher capacity to reveal polymorphisms and greater potential to determine intra and inter-genomic diversity than other arbitrary primer methods such as RAPD (Zietkiewicz *et al*., 1994). Thus, the genetic variation obtained from RAPD and ISSR analysis could be useful for selecting parents to generate the necessary cluster bean populations for genomic mapping and breeding purposes.

In the present study, RAPD and ISSR markers showed significant correlation (0.69), which implied random but highly correlated nature of both markers in exploiting genomic sites. The degree of fit can be interpreted as follows: $r \geq 0.9$, very good fit; $0.8 \leq r \geq 0.9$, good fit; $0.7 \leq r \geq 0.8$, poor fit; $r \leq 0.7$, very poor fit. High correlation between the two methods used in the present study proposes that either of the markers could be used for genetic diversity estimation in cluster bean. This correlation value for ISSR and ISSR+RAPD combined data was 0.89, while it was maximum for RAPD and ISSR+RAPD based similarities (0.93). Kafkas *et al*. (2006) reported a matrix correlation of 0.58 between ISSR and RAPD markers in their study. Lai *et al*. (2001) reported a matrix correlation of 0.811 between ISSR and RAPD cultivated tea clones and wild tea. Mattioni *et al*. (2002) utilized ISSR and RAPD markers to characterize 3 Chilean Nothofagus species and found matrix correlation of 0.95 between both markers.

The combined dendrogram of RAPD and ISSR data was more inclined towards RAPD-based dendrogram. It is probably because more polymorphic RAPD bands (147) were used to construct the combined dendrogram than the polymorphic ISSR bands (102). The results indicated that RAPD, ISSR and combined RAPD and ISSR analyses provided useful information on diversity among cluster bean genotypes.

Primer efficiency is an important parameter, especially in cases where random primers are used for genetic diversity analysis. Various statistical packages are available to calculate the primer robustness like marker index (MI) (Powell *et al*., 1996), diversity index (DI) (Weir, 1996), resolving power (Rp) (Prevost and Wilkinson, 1999), polymorphism information content (PIC) (Anderson *et al*., 1993), etc. However, discriminatory power (Dj), introduced by Tessier *et al*. (1999) is the most effective method to know the primer ability in distinguishing the crop genotypes using random markers. The *Dj* values for both RAPD and ISSR markers ranged from 0.40 to 0.98 (RAPD) and 0.44 to 0.99 (ISSR), respectively. The efficiency of the D_j is an extension of the PIC and provides an estimate of the probability that two randomly chosen individuals show different banding patterns for the same primer and, thus, are distinguishable from one another. Discriminatory power, a direct indication of primer efficiency, prevents the use of those primers which show less efficiency in distinguishing the genotypes. In the current set of genotypes, 5 out of 10 ISSRs showed high discriminatory power (> 0.90) while in

RAPD, only 6 out of 20 RAPD primers touched this index. Despite the great and similar discriminating power of both markers, some differences between the two could be detected: (1) Dj values lower for RAPDs than for ISSRs and (2) higher number of total, polymorphic and discriminant fragments for ISSRs than RAPDs. Although further investigations need to be made to confirm this observation, comparatively low polymorphism and less reproducibility of RAPD in the present study indicate ISSR as marker of the choice.

Analysis of genetic relationships in crop species, which provides information on planning the crosses between two diverse varieties, is the preliminary requirement for crop improvement strategy.The present set of cluster bean genotypes showed greater genetic diversity on the basis of random primers. However, the efforts of estimating genetic diversity among the present set of genotypes are not sufficient as only cultivated genotypes were included. Codominant markers such as SSRs are required for more information of genetic diversity of cluster bean by including more number of genotypes.

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تجزیه تنوع ژنتیکی ژنوتیپ های لوبیا خوشه ای [*Cyamopsis tetragonoloba* (L.) Taub] با کاربرد نشانگرهای راپید RAPD و ISSR

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چکیده

در این پژوهش، نشانگر های راپید و ISSR (inter-simple sequence repeat) برای تعیین تنوع ژنتیکی و روابط بین ۳۶ ژنوتیپ لوبیا خوشه ای [*Cyamopsis tetragonoloba* (L.) Taub] به کار رفتند. در تجزیه راپید۲۰ آغازگر تصادفی استفاده شد که ۱۶۴ نوار را تقویت(amplify) میکردند و ۱۴۷ نوار آنها چندشکلی بودند و دارای میانگین چندشکلی ۹۰/۳٪. اندازه محصولات تقویت شده بین ۲۵۰ تا bp ۲۰۵۰ بود. در تجزیه با نشانگر ISSR ، ۱۰ آغازگر به کار رفت که ۱۰۵ نوار تولید کردند واز میان آنها ۱۰۲نوار چند شکلی بودند(۹۷٪). اندازه نوارهای تقویت شده در این تجزیه بین ۴۵۰ تا ۳۵۰۰bp بود. به منظور تعیین کارآیی آغازگرها در تولید اطلاعات کافی برای تجزیه تنوع ژنتیکی، توان تمایز(Dj) محاسبه شد و مقدار آن بین ۰/۴ تا ۰/۹۸ برای راپید و بین ۰/۴۴ تا ۰/۹۹ برای ISSR به دست آمد. همچنین، از ضریب تشابه جاکارد(Jacard similarity coefficient) برای تعیین همبستگی ژنتیکی ژنوتیپ ها استفاده شد و تغییرات آن برای راپید بین ۰/۳۸ و ۰/۹۱ و برای ISSR بین۰/۲۰ و۰/۸۸ به دست آمد. تجزیه خوشه ای نشان داد که نشانگر های راپید و ISSR هر دو برای تشخیص و تمایزهمه ۳۵ ژنوتیپ قابل کاربرد بودند.هر دو روش مزبور همبستگی معنی داری نشان دادند(r= 0/69) که حاکی از اهمیت یکسان آنها در تجزیه تنوع و گوناگونی لوبیای خوشه ای بود.